Courtship Learning: Scent of a Woman

Learning to predict an outcome based on previous experience is of considerable selective advantage. Getting it wrong can be costly. In a complex environment, however, using the appropriate predictor is not necessarily a trivial task.

Scott Waddell

Researchers studying associative learning in *Drosophila* are in two camps. Some use a highly simplified learning protocol with tight experimenter control over two stimuli [1,2], to which they then add levels of complexity and adjust parameters [3,4]. Others use a naturally complex and more ethologically relevant protocol [5], and try to pare it down to minimal, preferably two, stimuli. In this issue of Current Biology, Ejima *et al*. [6] report a new paradigm that uncovers the intrinsic complexity of *Drosophila* courtship conditioning.

Fruit fly courtship behavior is a vigorous pursuit where the male chases the female and performs a number of stereotyped maneuvers, including vibrating a wing to play a species-specific courtship ballad [7]. These maneuvers are stimulated by signals from the female. In the lab, if a male fly is exposed to a previously mated female (which typically rejects his moves) for one hour, his subsequent advance toward another female is suppressed [5]. This male courtship suppression is called courtship conditioning. The memory can last for hours and is believed to depend in part on aversive pheromones emitted by the mated female [8]. Courtship learning is potentially multi-sensory. The male fly can see, smell, taste and touch the female and may or may not be successful in copulating.

Exposure to a mated female causes a suppression of subsequent courtship with immature, mature and mated female flies [5–6,9,10]. However, it makes little sense that a male fly would suppress all courtship activity after only one rejection. Clearly, there are many more flies waiting in the wings! It would appear profitable for a male fly to selectively avoid one female, or one type of female, while maintaining vigor toward another likely mate.

Ejima *et al*. [6] showed that males can in fact learn specifically to inhibit their courtship toward the type of female that they were previously rejected by. To do this they 'trained' males with a headless female of a certain maturity. The male flies (thankfully) never copulated with the decapitated female, and afterwards they displayed a maturation-specific courtship memory. If trained with a decapitated immature virgin, they preferentially avoided immature females over mature females and vice versa (Figure 1). They called this 'trainer-specific' memory. Interestingly, virgin females are not known to emit an aversive pheromone and therefore the mechanism of trainer-specific courtship suppression must differ from that induced by a mated female.

How can a male fly distinguish between females? Not all female flies look alike, but visual input is not critical for courtship [11]. It appears that females smell different. Flies have cuticular hydrocarbons that change in content and amount with maturity [6,12–14]. Ejima *et al*. [6] performed a chromatographic analysis of the cuticular hydrocarbons from females of differing age. Mature and immature flies have at least 63 common cuticular compounds and at least 25 different compounds. In general, total hydrocarbons increase as virgins mature and volatile alkenes are particularly affected.

In a series of crafty experiments, Ejima *et al*. [6] tested whether hydrocarbons represent volatile courtship relevant cues and maturity identifiers. The cuticular hydrocarbons can be transferred to filter papers by incubating flies with the papers for 1–4 hours, or extracted with hexane and spotted onto filters. The hydrocarbon impregnated filters alone do not modify subsequent courtship, suggesting that other cues are required. In fact, pairing a filter with a courtship object — a decapitated female of different maturity or even a male — reconstituted courtship memory.

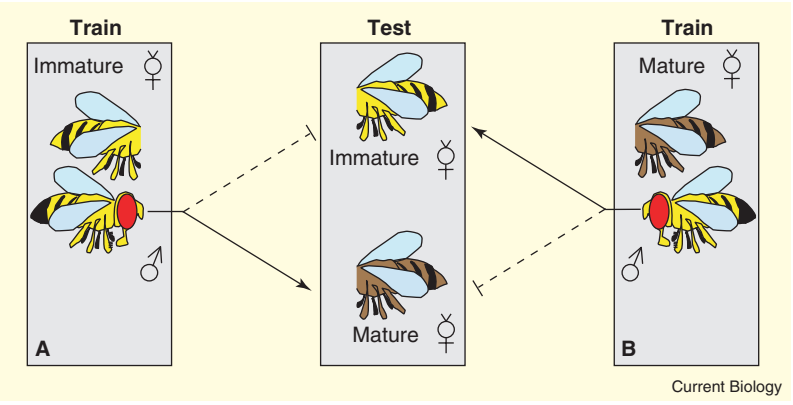

Figure 1. Trainer-specific courtship conditioning.
A single male is trained by exposure to a decapitated immature (A) or mature (B) virgin female fly for one hour. It is then tested for trainer-specific memory with either a decapitated immature virgin female or a decapitated mature virgin female. Training with an immature female will inhibit the male's courtship toward an immature tester female but not a mature tester female.

Ejima *et al*. [6] went to considerable length to show that trainer-specific courtship conditioning is associative. It requires a filter and a courtship object presented together. Presenting them sequentially, in any order, does not cause conditioning. Furthermore, some courtship learning is non-associative and results from habituation, but trainer-specific learning cannot be dishabituated.

It is likely, therefore, that trainer-specific learning is associative and requires a maturation specific hydrocarbon as a conditioned stimulus (CS) and a courtship object as an unconditioned stimulus (US). Ejima *et al*. [6] propose that failure to copulate is the US, because training with an intact female (which the male can copulate with) does not produce courtship memory, but training with a headless female (which the male fly does not copulate with) produces courtship memory.

Trainer-specific memory can be formed if the hydrocarbon-impregnated filters are separated from the courting males and courtship object with a mesh barrier, suggesting that the critical CS hydrocarbons are volatile and sensed by the olfactory system. Indeed, flies lacking olfactory organs are unable to form trainer-specific memory, but surprisingly they suppress courtship toward all females. Therefore male flies simultaneously learn multiple cues that have different salience, and in the absence of olfactory input they are left with a non-discriminatory courtship memory.

Ejima *et al*. [6] addressed whether male flies formed simultaneous memories by again pairing a mature female odor with an immature courtship object. Both of these cues have a distinctive hydrocarbon profile and this training produced courtship memories for immature and mature females. Remarkably, the authors were also able simultaneously to train flies to associate failure to copulate with a mature virgin and the odor benzaldehyde. Following training, males avoided mature virgins, but also immature virgins if they were tainted with benzaldehyde. Therefore the flies can associate multiple odor cues with failure to copulate and can use those memories to recognize and avoid courting an appropriately smelly female.

How do flies prioritize memories that are simultaneously formed? Some cues are likely to be more salient. Ejima *et al*. [6] showed that olfactory memories are dominant, because if the ability to smell is diminished, trainer-specificity is lost but a general courtship suppressing memory remains. In addition, some cue salience and memory priority will be determined by timing. Male flies can be sequentially taught two memories but, as time advances, the memory for the most recent encounter predominates. The authors speculate that an active process allows the second training session to disrupt consolidation of the first memory.

It will be very interesting to determine whether the known olfactory memory-relevant genes, brain anatomy and transmitter systems affect trainer-specific courtship memory. Ejima *et al*. [6] demonstrated that *amnesiac* mutant flies quickly lose their trainer-specific courtship memory, as they do in olfactory conditioning. More surprisingly, male flies carrying the *dunce*^{1} allele fail to show trainer-specific memory, but they have a general courtship memory like olfaction-defective flies.

Where are the maturity-specific memories? Do they require the mushroom bodies like other odor memories [15–17]? Are *amnesiac*-expressing DPM neurons important for the memory stability as they are in olfactory conditioning [18,19]? Does failure to copulate involve dopamine — like acquisition of negatively reinforced olfactory memory [20]? How do courtship memories affect the neuronal networks driving courtship behavior? Ejima *et al*. [6] found that conditioned males take longer to initiate courtship [6].

The perfume of a female fly looks complex. However, it should be possible to purify the hydrocarbons that differ between females and test their individual efficacy as cues. Do hydrocarbons individually signify maturity or is there a complex combinatorial perfume that identifies an individual fly? Clearly, the new trainer-specific memory model [6] asks many tantalizing questions. So far, we only have a sniff of the answers.

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DNA Ligase: Getting a Grip to Seal the Deal

The crystal structure of human DNA ligase I catches the enzyme just before the last step of ligation and shows that the protein wraps completely around nicked DNA. The elegant structure explains how ligase attains fidelity for the sealing operation.

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DNA ligation is the last step in a multitude of important DNA metabolic reactions [1]. DNA nicks introduced during replication, recombination and repair need to be sealed — if left unchecked, they would lead to cell death. So even before its discovery, a DNA ligase activity was proposed to exist. The discovery of ligase and the elegant biochemical studies of the ligase reaction by the Lehman group and others revealed the amazing series of chemical steps needed to seal a nick in DNA (reviewed in [2]). The beauty of ligase and its clever enzymatic strategy is highlighted by the recently reported crystal structure of human DNA ligase I (Lig1) bound to a nicked DNA substrate [3].

Sealing a nick in DNA might appear a simple task, but the reaction is exceedingly complex, requiring three distinct catalytic steps and two covalent intermediates (Figure 1A). The first step, enzyme adenylation, is accomplished using either NAD+ (in eubacteria) or ATP, but both result in an AMP-linkage to the enzyme. In the second step, the AMP moiety is transferred to the 5′ phosphate at the site of a nick. This activates the 5′ terminus for attack by the 3′ OH in the third and final phosphoryl transfer step, thereby sealing the nick.

Earlier ligase structures [4–7] were tremendously informative about the first step of the reaction and the AMP–ligase intermediate, and even suggested important features of the subsequent steps. The structure of Lig1 in a complex with nicked DNA [3] has now been determined, providing a three-dimensional snapshot of the moment before DNA ligation and giving conclusive insight into ligase fidelity and the final steps of the reaction. To form this stable reaction intermediate, Pascal *et al*. [3] used a synthetic nicked duplex terminated with a 3′ dideoxynucleotide, thereby removing the critical 3′ OH group necessary to form the phosphodiester bond. When Lig1 is reacted with this substrate in

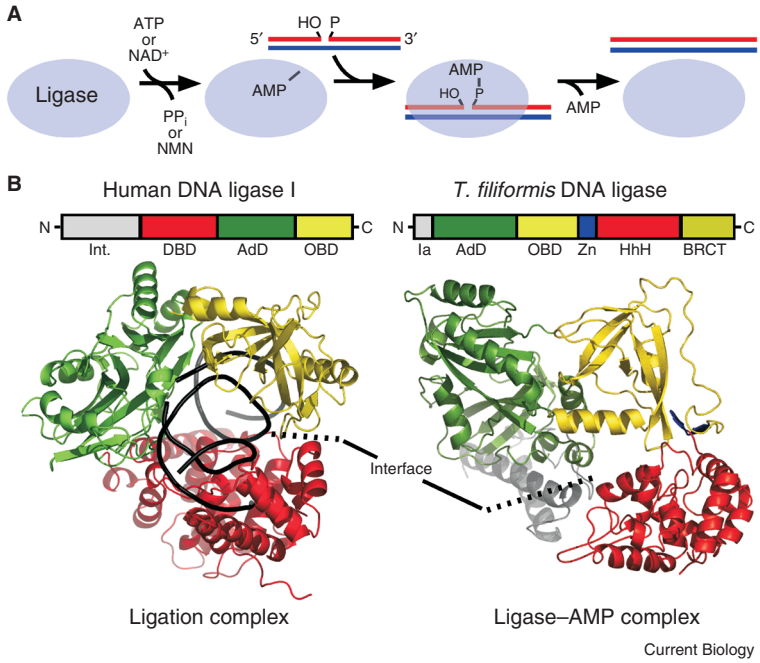

Figure 1. Comparison of prokaryotic and eukaryotic DNA ligase.

(A) Mechanism of DNA ligase. Note: bacterial cellular ligases use NAD+ and other ligases use ATP for self-adenylation. (B) DNA ligases have functionally similar domains, but they are scrambled in their linear sequence (top). Nevertheless, human Lig1 (left, in complex with DNA) and *Thermus filiformis* (*Tfi*) ligase (right) both adopt a ring-shaped structure (bottom). The gray interaction domain (Int.) of Lig1 was removed for crystal structure analysis. The helix-hairpin-helix (HhH) domain of *T. filiformis* ligase is analogous to the Lig1 DBD, but must undergo a large rotation with the OBD relative to the AdD for *T. filiformis* ligase to similarly accommodate DNA.